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Reviewer: Keisha Douglas

Timestamp: [year=2008; month=6; day=12; hr=19; min=27; sec=32; ms=818; ]

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Application No: 10566827

Version No: 1.1

Input Set:

Output Set:

Started: 2008-06-12 19:25:59.156

Finished: 2008-06-12 19:26:01.131

Elapsed: 0 hr(s) 0 min(s) 1 sec(s) 975 ms

Total Warnings: 20

Total Errors: 0

No. of SeqIDs Defined: 22

Actual SeqID Count: 22

Error code	Error Description
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W 213	Artificial or Unknown found in <213> in SEQ ID (21)
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**Input Set:**

**Output Set:**

**Started:** 2008-06-12 19:25:59.156  
**Finished:** 2008-06-12 19:26:01.131  
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**No. of SeqIDs Defined:** 22  
**Actual SeqID Count:** 22

Error code

Error Description

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# SEQUENCE LISTING

<110> de Lorenzo Prieto, Victor  
Fernandez Herrero, Luis A

<120> System for the Production of Dimeric Proteins Based on the  
Transport System of Hemolysin of Escherichia Coli

<130> 020884-000001

<140> 10/566,827

<141> 2006-01-31

<150> P200301830 (ES)

<151> 2003-07-31

<150> PCT/ES2004/070053

<151> 2004-07-19

<160> 22

<170> PatentIn version 3.4

<210> 1

<211> 36

<212> PRT

<213> Artificial

<220>

<223> EHlyA polypeptide containing 23 kDa ('hlyA) secretion signal of  
E. coli Hly transporter tagged with the E epitope.

<400> 1

Met Thr Met Ile Thr Asn Leu Asp Leu Asn Ser Val Ser Thr Pro Gly  
1 5 10 15

Gly Ala Pro Val Pro Tyr Pro Asp Pro Leu Glu Pro Ala Gly Glu Asn  
20 25 30

Ser Leu Ala Lys  
35

<210> 2

<211> 74

<212> PRT

<213> Artificial

<220>

<223> ZEHlyA polypeptide containing the 23 kDa ('hlyA) secretion signal  
of E. coli Hly transporter tagged with the E epitope.

<400> 2

Met Thr Met Ile Thr Asn Leu Asp Leu Asn Ser Val Ser Thr Ser Gly  
 1 5 10 15

Gly Pro Lys Pro Ser Thr Pro Pro Gly Ser Ser Arg Met Lys Leu Glu  
 20 25 30

Asp Lys Val Glu Glu Leu Leu Ser Lys Asn Tyr His Leu Glu Asn Glu  
 35 40 45

Val Ala Arg Leu Lys Lys Leu Val Gly Glu Arg Gly Gly His His His  
 50 55 60

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 65 70

<210> 3  
 <211> 949  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Ampicillin resistant plasmid pZEHlyA (sense strand); 23-kDa  
 C-terminal domain of HlyA with E-tag epitope incorporated at the  
 23-kDa C domain of HlyA

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 ttcgggtgtcg acgtccggcg gtccgaagcc ttccactcgg cccgggtctt cccgtatgaa 120  
 acagctggaa gacaaagtag aggagctcct tagcaagaac taccatctag aaaacgaggt 180  
 agctcgtctg aaaaagcttg ttggtgaacg tgggtggtcac catcaccatc accatgcgtc 240  
 gacgcccggg ggtgcgccgg tgccgtatcc ggatccgctg gaaccggccg gggaaaattc 300  
 tcttgctaaa aatgtattat ccggtggaaa aggtaatgac aagttgtacg gcagtgaggg 360  
 agcagacctg cttgatggcg gagaagggaa tgatcttctg aaaggtggat atggtaatga 420  
 tatttatcgt tatctttcag gatatggcca tcatattatt gacgatgaag gggggaaaga 480  
 cgataaactc agtttagctg atatagattt ccgggacggt gcctttaagc gagaagggaa 540  
 tgacctcatt atgtataaag ctgaaggtaa tgttctttct attggccaca aaaatggat 600  
 tacatttaaa aactggtttg aaaaagagtc agatgatctc tctaatacgc agatagagca 660  
 gatttttgat aaagacggca gggtaatcac accagattct cttaaaaaag catttgaata 720  
 tcagcagagt aataacaagg taagttatgt gtatggacat gatgcatcaa cttatgggag 780

ccaggacaat cttaatccat taattaatga aatcagcaaa atcatttcag ctgcaggtaa	840
cttcgatgtt aaggaggaaa gatctgccgc ttctttattg cagttgtccg gtaatgccag	900
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<210> 4  
 <211> 918  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Ampicillin resistant plasmid pZEHlyA (missense strand); 23-kDa C-terminal domain of HlyA with E-tag epitope incorporated at the 23-kDa domain of HlyA.

<400> 4	
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aggtgaggcg ggcccagaag ggcatacttt gtcgaccttc tgtttcatct cctcgaggaa	120
tcgttcttga tggtagatct tttgtccat cgagcagact ttttcgaaca accacttgca	180
ccaccagtgg tagtggtagt ggtacgcagc tgcgggcccc cacgcggcca cggcataggc	240
ctaggcgacc ttggccggcc ccttttaaga gaacgatttt tacataatag gccacctttt	300
ccattactgt tcaacatgcc gtcactccct cgtctggacg aactaccgcc tcttccctta	360
ctagaagact ttccacctat accattacta taaatagcaa tagaaagtcc tataccggtta	420
gtataataac tgctacttcc cccctttctg ctatttgagt caaatcgact atatctaaag	480
gccttgcaac ggaaattcgc tcttccctta ctggagtaat acatatttcg acttccatta	540
caagaaagat aaccggtgtt ttaccataa tgtaaatttt tgaccaaact ttttctcagt	600
ctactagaga gattagtagt ctatctcgtc taaaaactat ttctgccgtc ccattagtgt	660
ggtctaagag aatttttttcg taaacttata gtcgtctcat tattgttcca ttcaatacac	720
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tagtcgtttt agtaaagtcg acgtccattg aagctacaat tcctcctttc tagacggcga	840
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<210> 5  
 <211> 305  
 <212> PRT  
 <213> Artificial

<220>

<223> Ampicillin resistant plasmid pZEHlyA (protein); 23-kDa C-terminal domain of HlyA with E-tag epitope incorporated at the 23-kDa domain of HlyA.

<400> 5

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1 5 10 15

Gly Pro Lys Pro Ser Thr Pro Pro Gly Ser Ser Arg Met Lys Gln Leu  
20 25 30

Glu Asp Lys Val Glu Glu Leu Leu Ser Lys Asn Tyr His Leu Glu Asn  
35 40 45

Glu Val Ala Arg Leu Lys Lys Leu Val Gly Glu Arg Gly Gly His His  
50 55 60

His His His His Ala Ser Thr Pro Gly Gly Ala Pro Val Pro Tyr Pro  
65 70 75 80

Asp Pro Leu Glu Pro Ala Gly Glu Asn Ser Leu Ala Lys Asn Val Leu  
85 90 95

Ser Gly Gly Lys Gly Asn Asp Lys Leu Tyr Gly Ser Glu Gly Ala Asp  
100 105 110

Leu Leu Asp Gly Gly Glu Gly Asn Asp Leu Leu Lys Gly Gly Tyr Gly  
115 120 125

Asn Asp Ile Tyr Arg Tyr Leu Ser Gly Tyr Gly His His Ile Ile Asp  
130 135 140

Asp Glu Gly Gly Lys Asp Asp Lys Leu Ser Leu Ala Asp Ile Asp Phe  
145 150 155 160

Arg Asp Val Ala Phe Lys Arg Glu Gly Asn Asp Leu Ile Met Tyr Lys  
165 170 175

Ala Glu Gly Asn Val Leu Ser Ile Gly His Lys Asn Gly Ile Thr Phe  
180 185 190

Lys Asn Trp Phe Glu Lys Glu Ser Asp Asp Leu Ser Asn His Gln Ile  
195 200 205

Glu Gln Ile Phe Asp Lys Asp Gly Arg Val Ile Thr Pro Asp Ser Leu  
210 215 220

Lys Lys Ala Phe Glu Tyr Gln Gln Ser Asn Asn Lys Val Ser Tyr Val  
225 230 235 240

Tyr Gly His Asp Ala Ser Thr Tyr Gly Ser Gln Asp Asn Leu Asn Pro  
245 250 255

Leu Ile Asn Glu Ile Ser Lys Ile Ile Ser Ala Ala Gly Asn Phe Asp  
260 265 270

Val Lys Glu Glu Arg Ser Ala Ala Ser Leu Leu Gln Leu Ser Gly Asn  
275 280 285

Ala Ser Asp Phe Ser Tyr Gly Arg Asn Ser Ile Thr Leu Thr Ala Ser  
290 295 300

Ala  
305

<210> 6  
<211> 1979  
<212> DNA  
<213> Artificial

<220>

<223> Ampicillin resistant plasmid pZEHLA2SD (sense strand); 23-kDa  
C-terminal domain of HlyA with E-tag epitope incorporated at the  
23-kDa domain of HlyA and polylinker for cloning of scFv's in  
frame with E-tagged 'hlyA.

<400> 6  
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gagaccacaa cgggtttccct ctagaaataa ttttggttaa cttaagaag gagatatatc 120  
catggctagc acggcctcgg gggccgcgtc gacgtccggc ggtccgaagc cttccactcc 180  
gcccggttct tcccgtatga aacagctgga agacaaagta gaggagctcc ttagcaagaa 240  
ctaccatcta gaaaacgagg tagctcgtct gaaaaagctt gttggtgaac gtggtggtca 300  
ccatcaccat caccatgcgt cgacgcccg ggtgcgcgcg gtgccgtatc cggatccgct 360  
ggaaccggcc ggggaaaatt ctcttgctaa aaatgtatta tccggtggaa aaggtaatga 420  
caagttgtac ggcagtgagg gacgagacct gcttgatggc ggagaaggga atgatcttct 480



gaaaggtgga tatggtaatg atatttatcg ttatctttca ggatatggcc atcatattat	540
tgacgatgaa ggggggaaag acgataaact cagtttagct gatatagatt tccgggacgt	600
tgcctttaag cgagaaggga atgacctcat tatgtataaa gctgaaggta atgttctttc	660
tattggccac aaaaatggta ttacatttaa aaactggttt gaaaaagagt cagatgatct	720
ctctaatacat cagatagagc agatTTTTga taaagacggc agggtaatca caccagattc	780
tcttaaaaaa gcatttgaat atcagcagag taataacaag gtaagttatg tgtatggaca	840
tgatgcatca acttatggga gccaggacaa tcttaatcca ttaattaatg aaatcagcaa	900
aatcatttca gctgcaggta acttcgatgt taaggaggaa agatctgccg cttctttatt	960
gcagttgtcc ggtaatgcca gtgatttttc atatggacgg aactcaataa ctttgacagc	1020
atcagcataa tatattaatt taaatgatag caatcttact gggctgtgcc acataagatt	1080
gctatTTTTT tggagtcata atggattctt gtcataaaat tgattatggg ttatacgccc	1140
tggagatttt agcccaatac cataacgtct ctgttaacct ggaagaaatt aaacatagat	1200
ttgacacaga cgggactggc ctgggattaa cgtcatgggt gcttgctgcg aaatctttag	1260
aactaaaggt aaaacaggta aaaaaaaca ttgaccgatt aaactttatt tctctgcccg	1320
cattagtctg gagagaggat ggacgtcatt ttattctgac taaagtcagt aaagaagcaa	1380
acagatatct tatttctgat ctggagcagc gaaatccccg tgttctcgaa cagtctgagt	1440
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aactggcgaa atttgacttt acctggttta ttcctgccat tataaaatac aggagaatat	1560
ttattgaaac ccttgttgtg tctgtttttt tacaattatt tgcattaata accccccttt	1620
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ttactgtcgc attatctgtt gtggtggtgt ttgagattat actcagcggc ttaagaactt	1740
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atttactggc gctaccgatc tcttattttg agagtcgtcg tgttggtgat actgttgcca	1860
gggtaagaga attagaccag atccgtaatt ttctgacagg acaggcatta acatctgttc	1920
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<210> 7  
 <211> 1979  
 <212> DNA  
 <213> Artificial  
 <220>

<223> Ampicillin resistant plasmid pZEHLA2SD (missense strand); 23-kDa C-terminal domain of HlyA with E-tag epitope incorporated at the 23-kDa domain of HlyA and polylinker for cloning of scFv's in frame with E-tagged 'hlyA.

<400> 7

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gtaccgatcg tgccggagcc cccggcgagc ctgcaggccg ccaggcttcg gaaggtgagg	180
cgggcccgaga agggcatact ttgtcgacct tctgtttcat ctctcgagg aatcgttctt	240
gatggtagat cttttgctcc atcgagcaga ctttttcgaa caaccacttg caccaccagt	300
ggtagtggtg gtggtacgca gctgcggggc cccacgcggc caggcatag gcctaggcga	360
ccttgggccg ccccttttaa gagaacgatt ttacataat aggccacctt ttccattact	420
gttcaacatg ccgtcactcc ctgctctgga cgaactaccg cctcttcctt tactagaaga	480
ctttccacct ataccattac tataaatagc aatagaaagt cctataccgg tagtataata	540
actgctactt cccccctttc tgctatttga gtcaaatacg ctatatctaa aggccttgca	600
acggaaattc gctcttcctt tactggagta atacatattt cgacttccat tacaagaaag	660
ataaccggtg tttttaccat aatgtaaatt ttgaccaa ctttttctca gtctactaga	720
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acctctaaaa tcgggttatg gtattgcaga gacaattggg ccttctttta tttgtatcta	1200
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gtaatcagac ctctctccta cctgcagtaa aataagactg atttcagtca tttcttcgtt	1380
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aactccgcaa tatagtcctt gtataataag aatagcgaag ggcaagaaga caacggccct	1500
ttgaccgctt taaactgaaa tggaccaa ataggacggt atattttatg tcctcttata	1560

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aatgacagcg taatagacaa caccaccaca aactctaata tgagtcgcca aattcttgaa 1740  
tgtaaaaacg tgtatcatgt tcagcctaac tacaactcaa cccacggttt gagaaggccg 1800  
taaattgaccg cgatggctag agaataaaac tctcagcagc acaaccacta tgacaacggt 1860  
cccatctctt taatctgggc taggcattaa aagactgtcc tgtccgtaat tgtagacaag 1920  
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<210> 8

<211> 302

<212> PRT

<213> Artificial

<220>

<223> Ampicillin resistant plasmid pZEHLA2SD (protein); 23-kDa  
C-terminal domain of HlyA with E-tag epitope incorporated at the  
23-kDa domain of HlyA and polylinkerfor cloning of scFv's in  
frame with E-tagged 'hlyA.

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Met Ala Ser Thr Ala Ser Gly Ala Ala Ser Thr Ser Gly Gly Pro Lys  
1 5 10 15

Pro Ser Thr Pro Pro Gly Ser Ser Arg Met Lys Gln Leu Glu Asp Lys  
20 25 30

Val Glu Glu Leu Leu Ser Lys Asn Tyr His Leu Glu Asn Glu Val Ala  
35 40 45

Arg Leu Lys Lys Leu Val Gly Glu Arg Gly Gly His His His His His  
50 55 60

His Ala Ser Thr Pro Gly Gly Ala Pro Val Pro Tyr Pro Asp Pro Leu  
65 70 75 80

Glu Pro Ala Gly Glu Asn Ser Leu Ala Lys Asn Val Leu Ser Gly Gly  
85 90 95

Lys Gly Asn Asp Lys Leu Tyr Gly Ser Glu Gly Ala Asp Leu Leu Asp  
100 105 110

Gly Gly Glu Gly Asn Asp Leu Leu Lys Gly Gly Tyr Gly Asn Asp Ile  
115 120 125

Tyr Arg Tyr Leu Ser Gly Tyr Gly His His Ile Ile Asp Asp Glu Gly  
130 135 140

Gly Lys Asp Asp Lys Leu Ser Leu Ala Asp Ile Asp Phe Arg Asp Val  
145 150 155 160

Ala Phe Lys Arg Glu Gly Asn Asp Leu Ile Met Tyr Lys Ala Glu Gly  
165 170 175

Asn Val Leu Ser Ile Gly His Lys Asn Gly Ile Thr Phe Lys Asn Trp  
180 185 190

Phe Glu Lys Glu Ser Asp Asp Leu Ser Asn His Gln Ile Glu Gln Ile  
195 200 205

Phe Asp Lys Asp Gly Arg Val Ile Thr Pro Asp Ser Leu Lys Lys Ala  
210 215 220

Phe Glu Tyr Gln Gln Ser Asn Asn Lys Val Ser Tyr Val Tyr Gly His  
225 230 235 240

Asp Ala Ser Thr Tyr Gly Ser Gln Asp Asn Leu Asn Pro Leu Ile Asn  
245 250 255

Glu Ile Ser Lys Ile Ile Ser Ala Ala Gly Asn Phe Asp Val Lys Glu  
260 265 270

Glu Arg Ser Ala Ala Ser Leu Leu Gln Leu Ser Gly Asn Ala Ser Asp  
275 280 285

Phe Ser Tyr Gly Arg Asn Ser Ile Thr Leu Thr Ala Ser Ala  
290 295 300

<210> 9

<211> 2792

<212> DNA

<213> Artificial

<220>

<223> Ampicillin resistant plasmid pVamyHLYA (sense strand) containing  
amplified DNA product encoding VHH amylase (Vamy); 23-kDa  
C-terminal domain of HlyA with E-tag epitope incorporated at the

23-kDa domain of HlyA and polylinker for cloning of scFv's in

<400> 9

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gggtctctga	gactctectg	cacagcccct	ggattcacct	ccaatagctg	ccgcatggac	180
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cgcacaagct	atgcagactc	cgtgaagggc	cgattcacca	tctccaaaga	caaagccaag	300
gacacgggtg	atctgcaa	gaacagcctg	aaacctgagg	acacggccat	ctattactgt	360
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accaggtca	cgtctctctc	aacggcctcg	ggggccgcgt	cgacgcccgg	gggtgcgccg	480
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gctgaaggta	atgttctttc	tattggccac	aaaaatggta	ttacatttaa	aaactggttt	840
gaaaaagagt	cagatgatct	ctetaatcat	cagatagagc	agatttttga	taaagacggc	900
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